Stress induced by muzzle wearing in dogs

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Abstract
The study was conducted over a period of 6 months in collaboration with 2 private clinics in Iași on an initial number of 14 dogs of different breeds, sex and ages, clinically and paraclinically examined by hematological, biochemical, ultrasound and cortisol dosing, all being declared clinically healthy. The inclusion criterion in the study was the frequent wearing of the muzzle, all the individual being declared healthy prior to examination. The study aimed to establish the correlation between the increased level of sustained stress and the wearing of the muzzle in dogs with the possible development of adrenal gland neoplasia. The subjects were divided into 2 distinct groups, the first, consisting of 7 patients, represented by dogs that did wear muzzles, the second, 7 dogs who sis not wear it during testing. The test involved walking the dogs for about 60 minutes, four times in a week, under the same environmental and stimulation conditions for all participants, eventually being subjected to blood sampling to determine cortisol and to evaluate hematological changes at the beginning of the study, one and two weeks after. Compared with the initial values, group 1 showed significant increases of cortisol (from 3.76 ± 0.4 to 13.7±0.9 µg / dl), a marker of the stress level in the body. Also, the hematological picture revealed a decrease in eosinophil count. Subjects in group 2 had a slight increase in cortisol level (from 3,45±0,6 to 5.85±0.4 µg / dl), associated with no changes in total eosinophils. The study demonstrates the impact of muzzle wearing on dogs, which produces transitional changes in cortisol levels, but given the length of it, cannot be established any correlation with neoplasia development as the observed changes were only transitional.
Keywords: *stress, muzzle, dogs, cortisol*

Introduction

Cortisol is a steroid hormone that regulates a wide range of vital processes throughout the body, including metabolism and the immune response. It also has a very important role in helping the body respond to stress.[1]

Cortisol is a steroid hormone, one of the glucocorticoids, made in the cortex of the adrenal glands and then released into the blood, which transports it in the entire body. Almost every cell contains receptors for cortisol and so cortisol can have lots of different actions depending on which sort of cells it is acting upon. These effects include controlling the body's blood sugar levels and thus regulating metabolism, acting as an anti-inflammatory product, controlling salt and water balance and influencing blood pressure.[5]

The secretion of cortisol is mainly controlled by three inter-communicating regions of the body; the hypothalamus in the brain, the pituitary gland and the adrenal gland, the so called hypothalamic–pituitary–adrenal axis. When cortisol levels in the blood are low, the hypothalamus releases corticotrophin-releasing hormone, which causes the pituitary gland to secrete adrenocorticotropic hormone, into the bloodstream. High levels of adrenocorticotropic hormone are detected by the adrenal glands and stimulate the secretion of cortisol, causing blood levels of cortisol to rise.[3] As the cortisol levels rise, they start to block the release of corticotrophin-releasing hormone from the hypothalamus and adrenocorticotropic hormone from the pituitary. As a result, the adrenocorticotropic hormone levels start to drop, which then leads to a drop in cortisol levels, the regulation of cortisol being made by the means of a negative feedback loop.

Besides its main known functions, cortisol is a hormone that helps the organism progress on a daily basis. On the other hand, high levels of cortisol will overload the adrenal glands, with negative effects. Cortisol level is a well known marker of stress and togheter with cathecholamine discharges it may have a negative impact by means of neoplastic lessions development.[2,4]

The present study tried to assess if there are any correlations between stress induced by muzzle wearing and significant, sustained changes in the cortisol levels that may be considered a starting point in the development of adrenal gland neoplasia, given that in a 3 months period, 2 cases of pheochromocitoma were discovered in dogs usually wearing muzzles.

Material and methods

The study was conducted over a period of 6 months, between March-August 2019, in 2 private clinics from Iasi, on a total number of 14 dogs of different breeds, sex and ages, all of the subjects being used to muzzle wearing in order to avoid any further stress.

Dogs have been divided into 2 groups of 7 dogs each, individuals from the first group wearing muzzle thoughout the experiment and the other without wearing it. The experiment for each subject consisted in 4 walks of approximately one hour walk, the study for every dog lasting 2 weeks.

Inclusion criteria for this study was unremarkable hematology, biochemistry and ultrasound changes, with no functional or morphological changes that may affect cortisol levels.

Hematology was performed using Phoenix NCC-30 VET and BIOBASE BK-6200 automated hematological analyzers. Blood samples were collected either from the external saphenous vein or the jugular vein. The vacutainers contained EDTA, an anticoagulant substance and the samples were analyzed imediately after collecting them. For each case have been determined the following parameters: red blood cells (RBCs), packed cell volume (PCV), hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoblobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), WBC (white blood cells) and platelets. Also, a blood film was analyzed for each sample.

Biochemistry was performed in an accredited laboratory, having been determined glucose levels, liver enzymes, total protein and globulin, urea, creatinine and cortisol levels.

For ultrasound investigation a Mindray M9 ultrasound machine was used with a probe of 5-8 MHz.

Hematology, biochemistry and ultrasound investigations were performed at the beginning of the study, one week later - at the end of it and 2 weeks from the beginning in order to assess any permanent or transitional changes.

Results and discussion

Cortiso is produced from cholesterol in the two adrenal glands located on top of each kidney. Cortisol along with epinephrine is best known for its involvement in the “fight-or-flight” response and temporary increase in energy production, at the expense of processes that are not required for immediate survival. The resulting biochemical and hormonal imbalances resolve due to a hormonally driven negative feedback loop.[2]

High levels of cortisol over a long period of time have a negative impact on individuals, a well documented condition being Cushing`s disease. Endogenous hypercortisolism usually implies the presence of a pathologic condition caused by either an ACTH-secreting neoplasm or autonomous cortisol secretion from a benign or malignant adrenal neoplasm.[3] However, sustained or intermittent hypercortisolism may also accompany many medical disorders that stimulate

physiologic/non-neoplastic activation of the hypothalamic pituitary adrenal axis; these two entities sharing indistinguishable clinical and biochemical features. A thorough history and physical examination is often the best way to exclude pathologic/neoplastic hypercortisolism.[5]

Initial hematology, biochemistry and ultrasound data showed normal values for all individuals, all dogs being considered healthy prior the experiment.

Table 1 shows cortisol dosage results for both study groups. Basal cortisol levels at the beginning of the study were of 3,76±0,4 µg/dl (group 1) and 3,45±0,6 µg/dl (group 2). However, one week after the values showed an increase in both groups, 13.7±0.9 µg/dl (group 1) and 5.85±0.4µg / dl (group 2). There has been observed a very significant correlation between cortisol levels increase in individuals wearing muzzles and those not wearing it throughout the experiment, with $p<0.01$. Even if the changes were important during the experiment, two week from starting it cortisol levels dropped to 3,85±0,7 µg/dl (group 1) and 3,61±0,6 µg/dl (group 2).

Table 1. Cortisol levels at the beginning of the study (T_0), one week (T_1) and two weeks (T_2) after

Time of determination	Cortisol levels – group 1 µg/dl	Cortisol levels – group 2 µg/dl
T_0	3,76±0,4	3,45±0,6
T_1	13.7±0.9	5.85±0.4
T_2	3,85±0,7	3,61±0,6

The results show that only transitional changes have been recorded, changes also accompanied by an increase in blood glucose levels, from 86,3±0,5 mg/dl –group 1 and 82,8±0,4 mg/dl –group two (T_0) to 103,2±0,8 – group 1 and 96.9±0,6 – group two, at the end of the study (T_2). The increase in blood glucose levels support higher levels of cortisol identified in both groups when study ended.

As far as hematology results concerned, only eosinophils values suffered a decrease as a stress response observed in group 1 individuals, eosinopenia being usually seen in stressed organisms. No hematological changes were seen for group 2 dogs.

Ultrasound did not reveal any changes, as expected, given the short period of experiment.

The body's stress-response system is usually self-limiting. Once a perceived threat has passed, hormone levels return to normal. As adrenaline and cortisol levels drop, the body will return to its regular activities.[4] On the other hand, when stressors are always present and the body constantly feels under attack, that fight-or-flight reaction stays turned on. The long-term activation of the stress-response system and the overexposure to cortisol and other stress hormones that follows can disrupt almost all of body's processes. This puts it at increased risk.

However, the conducted study showed that the changes that have been observed are only transitional, after removing the stress agent, the levels of cortisol dropping to normal values.

Conclusions

The study revealed a significant increase of cortisol levels in individuals wearing muzzles on a regular basis, with $p<0,01$. Nevertheless, the changes did not last after removal of the stress agent and given the short extend of the experiment, no correlation between stress associated to muzzle wearing and development of neoplastic lessions in dogs can be observed.

The study recommends however an extended experimental period but again raises some questions about owners willingness to accept a prolonged stress response from their pets.

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